Journal of Toxicology TOXIN REVIEWS Vol. 23, Nos. 2 & 3, pp. 317-344, 2004

Emerging Technologies for Mycotoxin Detection

Chris M. Maragos*

Agricultural Research Service, United States Department of Agriculture, National Center for Agricultural Utilization Research, Peoria, Illinois, USA

CONTENTS

	ABSTRACT	. 3	318
I.	INTRODUCTION	. 3	318
Π.	SENSORS FOR DETECTING FUNGAL TOXINS		
	1. Fiber Optic Devices		
	2. Surface Plasmon Resonance	. 3	324
	B. Lateral Flow and Dipstick Devices	. 3	327
	C. Fluorescence Polarization and Time-Resolved Fluorescence.		

317

DOI: 10.1081/TXR-200027859 Copyright © 2004 by Marcel Dekker, Inc. 0731-3837 (Print); 1525-6057 (Online) www.dekker.com





^{*}Correspondence: Chris M. Maragos, Agricultural Research Service, United States Department of Agriculture, National Center for Agricultural Utilization Research, 1815 N. University St., Peoria, IL 61604, USA; E-mail: maragocm@ncaur.usda.gov.

ABSTRACT

The history of the development of analytical methods for detecting fungal toxins is rich and varied. Method development has followed a process somewhat akin to Darwinian evolution: methods are selected based upon the characteristics most desirable to the analyst. Typically, this has lead to the development of accurate and sensitive methods for their detection, with a recurring emphasis on improving the speed and lowering the costs of the assays. Like evolution, there have been radical developments, incremental developments, and techniques that have fallen from favor only to be rediscovered. This review focuses on recent developments in technologies for detection of mycotoxins, with a particular emphasis on the myriad forms of biosensors that have begun to appear. Specifically, recent development in evanescent wave technologies (surface plasmon resonance, fiber optic sensors), lateral flow and dipstick devices, fluorescence polarization and time-resolved fluorescence, microbead assays, and capillary electrophoretic immunoassays, are described. The challenge for the emerging technologies is to demonstrate advantages over the more conventional, and better established, techniques in settings outside the analytical laboratory.

Key Words: Mycotoxin; Analysis; Immunoassay; Biosensor.

I. INTRODUCTION

Since the link was first made between chemical agents produced by fungi and disease in animals, technologies for detection of these mycotoxins have steadily advanced. As new analytical technologies have developed they have been rapidly incorporated into mycotoxin testing strategies. The reasons for this rapid adaptation have been the desire to protect human health, the economic incentives of protecting livestock from acute toxicity, the gains in production obtained from reducing the chronic effects of exposure to mycotoxins, and the need to test in order to meet contract specifications for minimum acceptable levels in foods and feeds. These forces have lead to the continued experimentation and improvement in mycotoxin



analytical testing. This review will focus upon emerging technologies that may, in the future, find use in the monitoring of mycotoxins in agricultural commodities and foods.

Detection of mycotoxins themselves must be distinguished from the detection of mycotoxin-producing fungi. Historically, the fungi have been detected either visually (i.e., moldy grain) or indirectly by the effects they cause on foodstuffs as they grow. The growth of fungi within a foodstuff changes the physical and chemical composition of the food, and these changes can often be used advantageously to distinguish fungally infected material. Removing the bulk of fungal contamination can be expected to reduce the mycotoxin content of the material, provided the fungus is mycotoxigenic. Early studies used the bright greenish-yellow fluorescence (BGYF)^a of a kojic acid derivative as a marker for aflatoxin contamination. The effect was the basis for a fiber optic device for detecting BGYF in corn kernels (McClure and Farsaie, 1980). More recently, image analysis, machine vision systems, and infrared spectroscopy have all been used to detect the changes wrought by fungal infection (Dowell et al., 1999, 2002; Hirano et al., 1998; Kos et al., 2002; Pearson et al., 1996, 2001; Ruan et al., 1998). The advantages of such techniques are that they may eventually allow the rapid sorting of commodities based upon fungal content. This, in turn, could be used as a tool to reduce exposure to the toxins. As such the newer fungal detection technologies hold considerable promise. Nevertheless, the indirectness of the association of fungal content with mycotoxin content necessitates the detection of the fungal toxins themselves. The remainder of this review will focus on methods for the detection of mycotoxins.

Testing for mycotoxins is conducted under many different circumstances and for a variety of reasons, which has led to a proliferation in the number of test methods. Selecting the appropriate method depends upon the intended use for the method. Factors such as the speed of the method, its accuracy, the skill level required to perform the assay, and the cost will all impact upon method selection. The methods basically fall into two major categories: those that can be conducted with minimal training in portable laboratories or the field (screening assays), and those that must be



^aAbbreviations used: aflatoxin B_1 (AFB₁), aflatoxin G_1 (AFG₁), aflatoxin M_1 (AFM₁). bright greenish-yellow fluorescence (BGYF), bovine serum albumin (BSA), capillary electrophoresis (CE), deoxynivalenol (DON), enzyme-linked immunosorbent assay (ELISA), fluorescein (FL), fluorescence polarization (FP), fumonisin B₁ (FB₁), flow injection liposome immunoanalysis (FILIA), immunoaffinity column (IAC), ochratoxin A (OA), sequential injection immunoassay (SIIA), surface plasmon resonance (SPR), time resolved fluoroimmunoassay (TR-FIA), zearalenone (ZEN).

conducted by more fully trained personnel in analytical laboratories. In all cases, obtaining a representative test sample of the overall lot is essential in order to ensure that the results of the test sample can be correctly ascribed to the lot. Sampling, subsampling, grinding, and extraction of commodities takes considerable attention and time. In fact, these steps often require more time than some of the rapid assays for detecting the toxins. Therefore, where possible, it is preferable to combine a rapid extraction technique with rapid assays in order to minimize the overall assay time.

Widely used methods include some of those that were developed when mycotoxins were first identified as chemical agents, such as thin layer chromatography (TLC). The development of antibodies to the major mycotoxins in the 1970s and 1980s led to the increased use of enzymelinked immunosorbent assays (ELISAs). The ELISAs are extensively used as screening methods, and over time commercial ELISA test kits have been developed, many of which have been validated through organizations such as the Association of Official Analytical Chemists (AOAC) International. The website of the AOAC International (www.aoac.org) is a good resource for locating commercially available test kits. Refinements to ELISA technology continue, including the development of more sensitive amplification mechanisms (Bhattacharya et al., 1999).

Chromatographic methods for mycotoxins have continously been developed and improved. Commonly used liquid chromatographic methods include high-performance liquid chromatography (HPLC) with fluorescence detection for aflatoxins, zearalenone, ochratoxins, and (derivatized) fumonisins, and HPLC with ultraviolet (UV) detection for deoxynivalenol. Many of the trichothecene mycotoxins are commonly analyzed by gas chromatography (GC) with various detectors. This review cannot adequately address the nuances of the many chromatographic methods, and interested readers are directed to excellent reviews by Sydenham and Shephard (1997), Shephard (2001), and Trucksess (2001). Thin layer chromatography, once a mainstay of mycotoxin analysis, has been supplanted by HPLC in many locations. However, given the significant advantages of the low cost of operation and the potential to test many samples simultaneously, and advances in instrumentation that allow quantification by image analysis or densitometry (Karuna and Sashidhar, 1999; Stroka and Anklam, 2000), TLC remains a viable screening technique for mycotoxins.

Because of the advantages of specificity and selectivity, chromatographic separation combined with mass spectrometric (MS) detection has continued to expand as a laboratory-based method for detecting mycotoxins. The HPLC-MS methods for all of the major, and many of the minor



mycotoxins have been published, and continue to be developed and improved (Cappiello et al., 1995; Hartl and Humpf, 1999; Musser et al., 2002; Plattner and Maragos, 2003; Tanaka et al., 2002; Zöllner et al., 1999). Clearly, the use of mass spectrometric methods can be expected to increase, particularly as they become easier to use and the costs of instrumentation continues to fall.

Despite the considerable advantages of the chromatographic techniques, most remain laboratory-based assays because they require considerable skill and/or instrumentation to operate. On the other end of the analytical spectrum are the immunoassays: assays that use the inherent specificity of antibodies to bind target analytes. By far the majority of reported mycotoxins are low molecular weight (less than 1000 daltons), which restricts somewhat the formats of immunoassays that can be used for their detection. Most, but not all, mycotoxin immunoassays are competitive immunoassays of one of two types, either (A) so-called "direct" assays where the mycotoxin-specific antibody is attached to a surface or (B) socalled "indirect" assays where a mycotoxin or mycotoxin-protein conjugate is attached to a surface (Fig. 1). The terminology is somewhat misleading, because both formats entail measurement of an enzymatic product, not direct detection of the mycotoxin itself. The distinction between whether antibody or antigen is attached to the surface is important and has implications for the formats of biosensors, as will be discussed below.

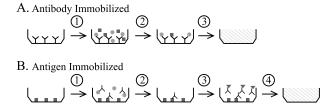


Figure 1. Two common immunoassay formats using either immobilized antibody or immobilized antigen. (A) With antibody (Y) immobilized the steps are: (1) addition of sample (toxin: ●) and toxin-enzyme conjugate (■) and incubation, (2) wash to remove unbound conjugate, and (3) addition of substrate, incubation, measurement of colored product. (B) With antigen immobilized (1) the steps are: (1) addition of sample (toxin: ●) and anti-toxin antibody (Y) and incubation, (2) wash to remove unbound antibody, (3) addition of secondary antibody-enzyme conjugate (Y▼) and incubation, (4) wash to remove unbound conjugate, addition of substrate, incubation, measurement of colored product.



II. SENSORS FOR DETECTING FUNGAL TOXINS

Many technologies exist for detecting low molecular weight materials. Those that have recently been applied to mycotoxin detection include: evanescent wave technologies, lateral flow and dipstick devices, fluorescence polarization, microbead assays, flow injection liposome immunoassays, capillary electrophoretic immunoassays, and flow injection lipid bilayer assays.

A. Evanescent Wave Technologies

1. Fiber Optic Devices

When light is applied to some materials, such as optical fibers, it can, under the appropriate conditions, undergo a process known as total internal reflection. When this occurs essentially all of the light that is applied (launched) to the surface is propagated through the fiber. However, a small portion of the applied light exits the fiber perpendicular to it in the form of an evanescent wave. The evanescent wave can also be formed when light is reflected off other surfaces, such as glass slides. The characteristics of the evanescent wave are influenced by the refractive index of the fiber, the refractive index of the surrounding material, and the incident light. A useful property of the evanescent wave is that its intensity decreases exponentially with distance from the interface between the fiber and the surrounding material (Thompson and Ligler, 1991). There are several ways in which this effect can be used. The first of these is with fiber optic devices, where a binding event can be made to occur near the surface of the fiber. The second is with the induction of plasmons in a metal film, as in surface plasmon resonance.

With fiber optic-based devices there are several forms by which the binding event can be monitored: analogous to the situation discussed above with ELISAs (Thompson and Ligler, 1991). That is, either antibody or antigen can be attached to the surface of the fiber. Examples with mycotoxins include immunosensors for fumonisins and aflatoxins. In the former case, antibodies to fumonisin were attached to the surface of optical fibers. The fibers were then exposed to sample mixed with fluorescently labeled fumonisin B₁ (FB₁-FL). The labeled and unlabeled FB₁ competed for binding to the surface of the fiber. After a washing step to remove unbound fluorophore, the amount of bound fluorophore was detected. This was possible because a small proportion of the fluorescence emission of the FB₁-FL was captured by the optical fiber, where it was transmitted to a sensitive detector. This device was capable of detecting



fumonisin B_1 in maize samples within 8 min with moderate sensitivity, having a midpoint for the calibration curve (IC₅₀) of 70 ng FB₁/mL (Thompson and Maragos, 1996). The technique was applied to corn samples extracted with methanol/water. Simple dilution of the methanolic extract, in order to reduce the methanol content, gave an assay with poor sensitivity, having an IC₅₀ of 25,000 ng FB₁/g maize. Concentrating the FB₁ with an immunoaffinity column improved the sensitivity of the assay, but negated the primary advantage of the method, speed (Maragos and Thompson, 1999).

A similar device was constructed for aflatoxin B₁ (AFB₁) using antiaflatoxin antibodies attached to the optical fiber (Maragos and Thompson, 1999). Unlike fumonisins, the aflatoxins have a native fluorescence that could be monitored. The binding of aflatoxin to antibody increases the concentration of the fluorophore at the surface of the fiber, effectively resulting in a noncompetitive assay for aflatoxin (vs. the competitive format of the fumonisin assay). The major advantage of the noncompetitive format would be direct, rather than indirect, detection of the toxin. The fiber optic device detected aflatoxin B₁, with a limit of determination of 2 ng AFB₁/mL. However, small changes in refractive index of the solution surrounding the fiber (i.e., sample) greatly affected the size of the evanescent wave, and therefore the response.

A fluorescence-based immunosensor for AFB₁ that did not use the principle of evanescence has also been reported (Carter et al., 1997). In this case, aflatoxin antibodies were immobilized to a membrane. The membrane was exposed to AFB₁ for 1 hour then washed and fitted onto the tip of an optical fiber connected to a fluorometer. The fluorescence of bound AFB₁ was then detected, over the range of 0.05 to 20 ng/mL. Samples of corn and peanuts were tested after cleanup. Corn samples were extracted with methanol/water, treated with NaCl/zinc acetate, partitioned with benzene, dried, and reconstituted before testing. Peanut samples were extracted with acetonitrile/buffer overnight then centrifuged and the supernatant used directly. The limit of detection in buffer, peanut extract, and corn extract was 0.05 ng/mL. The same article also described two types of competitive assay where an aflatoxin-bovine serum albumin conjugate (AFB₁-BSA) was coated on the membranes. In both formats, free AFB₁ competed with bound AFB₁-BSA for aflatoxin antibody binding. In the first format the aflatoxin antibody was unlabeled but was detected with a secondary antibody labeled with the enzyme alkaline phosphatase. An additional rinsing step was added to remove the excess secondary antibody before fitting the membrane onto the sensor. The amount of secondary antibody was then determined after the addition of a fluorogenic substrate. The limit of detection was 5 ng/mL in peanut extract. In the second format the aflatoxin antibody was itself



labeled with fluorescein, so addition of substrate was not needed. The limit of detection was 1 ng/mL in peanut extract.

2. Surface Plasmon Resonance

The evanescent wave effect has also been applied using the phenomenon of surface plasmon resonance (SPR; Liedberg et al., 1995). With SPR, light is used to excite plasmons (electron charge density waves) in a thin film of gold foil attached to the surface of a glass prism (Fig. 2). The resonance is a coupling between the light energy and the surface plasmons in the gold film. When the resonance occurs there is a resultant absorption of energy and a decrease in the intensity of the reflected light. The angle of incident light at which this process occurs is known as the resonance angle (θ) in Fig. 2), and it is dependent upon the refractive indices of the prism and the sensor surface. If the incident light is applied in a fashion such that there are multiple incident angles (i.e., as shown in Fig. 2 the incident light is a wedge), then the characteristic drop at the resonance angle creates what amounts to a shadow within the reflected light (Pharmacia Biosensor AB, 1995).

The evanescent wave extends outward from the surface of the gold foil into the surrounding solution. The intensity of the evanescent wave decays exponentially with distance from the surface. Materials that interact near the surface alter the refractive index of the surface, thereby changing the resonance angle. The SPR sensors measure binding phenomena by detecting

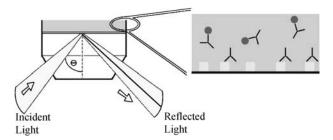


Figure 2. Schematic of a surface plasmon resonance biosensor for mycotoxins. The left panel depicts the incident light applied through a prism onto a glass slide, reflection of the light and formation of an evanescent wave. In the format shown a mycotoxin or mycotoxin-protein conjugate (antigen) is attached to the surface of the sensor chip and antibody binding is detected. Competition between the immobilized antigen (■) and free toxin (●), from the sample, decreases the amount of antibody (Y) bound at the surface of the chip.



either the change in the resonance angle or the change in intensity of the reflected light. In either case the magnitude of the response is affected by the amount (mass) of material that adheres to the sensor surface. The SPR sensors then, in a fashion, act as mass sensors. Devices using this technology are available commercially from several sources, but can also be fabricated.

The formats for the SPR immunoassays mimic those of the ELISAs: namely, either antigen is attached to the surface or antibody is attached to the surface, analogous to the illustration in Fig. 1. The schematic in Fig. 2 illustrates the case where mycotoxin or a mycotoxin-protein conjugate (antigen) is attached to the surface of the chip, and the binding of mycotoxin-specific antibody is detected in a competitive assay. The alternative format is the attachment of the mycotoxin-specific antibody to the surface of the chip. In the latter case, the binding of toxin to the antibody can be measured in a noncompetitive assay.

Assays for mycotoxins using both formats have been described, although most have used antigen-modified surfaces to detect antibody binding. The first group to report assays of this form for mycotoxins was TNO in the Netherlands (van der Gaag et al., 1996). The SPR assays have been described for AFB₁, deoxynivalenol (DON), zearalenone (ZEN), ochratoxin A (OA), and fumonisins. The majority of SPR applications for mycotoxins have used instrumentation commercially available from Pharmacia AB (Uppsala, Sweden). AFB₁ was detected by SPR using a format with AFB₁-BSA antigen attached to the sensor surface and competition between the bound antigen and AFB₁ in solution (Daly et al., 2000). Two polyclonal antibody preparations were tested. AFB₁ standards were incubated with the aflatoxin antibodies for 10 min before being applied to the sensor. After an interval during which the antibody attached to the sensor surface, the antibody/antigen interaction was disrupted with 1 M ethanolamine in 20% (v/v) acetonitrile in order to regenerate the surface of the sensor. The sensor had a linear range of detection between 3 and 98 ng AFB₁/mL, which was slightly more sensitive than the same antibody preparation tested in an ELISA format (linear range between 12-25,000 ng/mL).

Because SPR measures antibody/antigen interaction, the technique can be used to select for high-affinity antibodies or antibody fragments. This approach was taken for selection of single-chain antibody fragments for binding to AFB₁-BSA (Moghaddam et al., 2001). Two phage-display antibody libraries were panned against AFB₁-BSA and the antibody fragments were screened for binding to AFB₁-BSA by SPR. The technique allowed the isolation of single-chain fragment variable (scFv) antibody domains with affinity for AFB₁. The scFv were then evaluated in a



competitive SPR assay, and although the limits of detection were not specified, the assay was shown as able to detect a 50-nM solution of AFB₁ or AFG₁ (circa 16 ng/mL). Anti-aflatoxin scFv have also been used for detection of AFB₁ in spiked extracts of grain (Daly et al., 2002). The range of detection for the assay was between 3 and 195 ng AFB₁/mL in phosphate-buffered saline and 0.75 to 48 ng/mL in spiked grain extracts. The type of grain used was not specified. The enhanced performance of the assay in the grain extracts may be due to the presence of 5% (v/v) methanol in the extracts, a situation also known to occur with some ELISAs for AFB₁.

Deoxynivalenol has also been detected using SPR (Schnerr et al., 2002; Tüdos et al., 2003; van der Gaag et al., 2003). Deoxynivalenol was attached to the surface of the sensor using a DON-biotin conjugate and a streptavidin-coated sensor surface (Schnerr et al., 2002). Wheat was extracted with aqueous methanol, centrifuged, and the extract passed through a solid-phase extraction column (Romer MycoSep) to remove impurities. The purified extract was combined with a rabbit polyclonal antibody before injection into the sensor. Greater response could also be attained by adding a secondary, antirabbit, antibody. Using the secondary antibody increased the signal 5-fold, but also shifted the working range toward higher concentrations. The assays were relatively rapid, with a 10min sample preparation time and a 5-min analysis time. Recovery of DON from wheat spiked at 50 to 500 ng/g averaged $104\% \pm 15\%$. The working range was between 130 and 10,000 ng DON/mL (equivalent to 390 to 3000 ng/g in samples), with an IC₅₀ of 720 ng/mL. Results by SPR were correlated with results from GC-MS and HPLC-UV of contaminated wheat.

Recently, Tüdos et al. (2003) reported an assay for DON in wheat using SPR with a DON-casein conjugate immobilized on the sensor surface. After the antibody binding step, the sensor surface was regenerated with 6 M guanidine chloride, and the sensor could be used 500 times without significant loss of activity. The assay was used to detect DON in wheat extracted with acetonitrile/water. No sample cleanup was necessary; however, the samples needed to be diluted before injection in order to reduce the solvent strength to minimize the affect on the DON antibodies. The assay had a working range of 2.5 to 30 ng DON/mL, and results correlated well between the sensor and HPLC-MS-MS for eight wheat samples.

A technique for the detection of up to four mycotoxins simultaneously using SPR was recently reported (van der Gaag et al., 2003). The fluidics of the instrument, a BIACORE 2000, were constructed so that the sensor surface contained four serially connected flow cells. As with the previously described SPR assays, sample was mixed with mycotoxin-specific antibodies before injection onto the sensor. Instead of immobilizing toxin-protein conjugates onto the sensor surface the toxins



themselves were immobilized. The exception was for DON, which was immobilized as a DON-BSA conjugate. Samples were extracted with 90% (v/v) acetonitrile/water and a portion of the extract cleaned up with a solid phase extraction column (Romer MycoSep 224 MFC Romer Labs, Union, MO), which bound impurities and allowed the toxins to pass through into the filtrate. The filtrate was diluted 10-fold and mixed with the four antibodies (ZEN, DON, FB₁, AFB₁) before assay. Assays could be conducted within 25 min, including the sensor regeneration time.

The second SPR format: using antibody-modified surfaces to directly detect toxin binding noncompetitively has been reported for the fumonisins (Mullett et al., 1998). It is possible with the fumonisins because of their larger molecular weight (721 daltons for FB₁) relative to other mycotoxins such as aflatoxin (312 daltons). Polyclonal antifumonisin antibodies were immobilized on the sensor surface. The latter assay had a limit of detection of standard solutions of FB₁ of 50 ng/mL and could be conducted in 10 min. Application of the assay to fumonisins in foods was not reported. The advantage of the immobilized antibody format is that it is noncompetitive; therefore, the response was directly proportional to the amount of toxin present. A second advantage is that a toxin-protein conjugate was not required.

B. Lateral Flow and Dipstick Devices

A long-desired format for mycotoxin assays is one analogous to home pregnancy test kits in which the presence of the mycotoxin is directly detected in a rapid disposable device. Devices of this type are also known as immunochromatographic tests. A major impediment to the development of kits of this format for mycotoxins has been their low molecular weight. Unlike the detection of larger antigens, where multiple antibodies can be attached and used to make noncompetitive "sandwich" formats, the detection of low molecular weight toxins has relied upon competitive assays. A disposable device that uses a membrane-based flow-through immunoassay has been available commercially for many years for detection of aflatoxin M₁ (AFM₁) in milk (AflaCup, International Diagnostic Systems Corp., St. Joseph, MI). With the AflaCup format the applied liquids flow through the membrane and are collected on an absorbent pad on the opposite side of the membrane. The label is enzymatic, which requires that a substrate-incubation step be included. This type of assay is also known as an enzyme-linked immunofiltration assay (ELIFA). A flow-through membrane immunossay for detection of ochratoxin A in wheat was also reported (De Saeger and Van Peteghem, 1999), and a collaborative study of kits to detect OA and T-2 toxin was conducted (De Saeger et al., 2002).



The limits of detection were 4 ng/g and 50 ng/g for OA and T-2, respectively, in wheat, rye, maize, and barley.

Lateral flow devices differ from ELIFA in that the flow is directed laterally across the membrane rather than through it (parallel rather than perpendicular flow), analogous to thin-layer chromatography. Lateral flow devices can be made in several forms depending upon which reagent is labeled (the toxin or the antibody) and the form of the label (enzymatic, labeled liposomes, or colloidal gold). A lateral flow device using an enzymatic marker, a lateral flow ELISA, was sold commercially for aflatoxins, OA, T-2 toxin, and ZEN by Editek (Burlington, NC).

A dipstick assay similar to a lateral flow device was developed for FB₁ in corn-based foods and was reported to have a visual limit of detection of 40-60 ng FB₁/g sample (Schneider et al., 1995a). In this format the test strips had two lines: one line corresponded to antifumonisin antibody and the other to antihorseradish peroxidase antibody. Test strips were incubated in a tube containing the tracer (fumonisin-horseradish peroxidase) and the sample extract. After washing and addition of substrate, the two lines were visualized. The anti-HRP line acted as a positive color control, while the presence of FB₁ was indicated by a reduction in the color at the fumonisin antibody line. Assay time was about 1 h. A similar type of device was developed for T-2 toxin in wheat (De Saeger and Van Peteghem, 1996).

An interesting adaptation of the aforementioned dipstick format was the application to the detection of multiple mycotoxins in wheat (Schneider et al., 1995b). Using multiple mycotoxin-specific antibodies and multiple toxin-HRP conjugates, researchers were able to detect aflatoxin B₁, T-2 toxin, 3-acetyl-DON, roridin A, and ZEN. In the latter publication, the response of the test (toxin-exposed) dipstick was compared to the response of a control dipstick (not exposed to toxin) for estimation of toxin presence. The detection limits for spiked wheat samples were 30 ng/g, 100 ng/g, 600 ng/g, 500 ng/g, and 60 ng/g for AFB₁, T-2, 3-acetyl-DON, roridin A, and ZEN, respectively.

Commercial lateral flow devices for mycotoxins continue to be developed, with the goals of combining the negative control reaction on the same strip as the sample, and shortening the required assay time. Interesting variations include the possibilities of using labeled liposomes or colloidal gold conjugates to avoid the enzymatic reaction step. A lateral flow device was constructed for aflatoxin B₁ using aflatoxin-modified liposomes (Ho and Wauchope, 2002). The liposomes contained sulforhodamine B, a visible (and fluorescent) dye. Aflatoxin-specific antibody was attached to the strip and the strip exposed to AFB₁. The labeled liposomes were then added and competed for binding to the antibody, with the color at the site of the antibody inversely proportional to the AFB₁



level. Assays times were 12 min. The device could detect as little as 20 ng AFB₁, although on a concentration basis the detection was relatively poor, with the midpoint of the binding curve occurring at approximately 2000 ng/mL. Unfortunately, the liposomes also showed some instability to solvents. Notwithstanding this result, the assay demonstrates the possibility of shortening analysis times through the use of nonenzymatic labels.

Because of the ease of use of these devices, efforts to develop dipstick and lateral flow assays for mycotoxins are likely to continue, particularly with stable, nonenzymatic labels. Recently, American Bionostica (Logan Township, NJ) in collaboration with R-Biopharm (Darmstadt, Germany) developed lateral flow devices for DON, fumonisins, and aflatoxins. Grain samples were extracted with buffer and the extract allowed to settle for 5-10 min. Several drops of the extract were then transferred to the test device (cassette). The test strips contain two lines, one of which is a negative control. Positive results are indicated by the decrease of intensity of red color at the test line. Further information on the devices can be obtained from R-Biopharm. Lateral flow test strips have also been developed by Charm Science, Inc. (Lawrence, MA). The strips measure AFM₁ in milk, and use a portable hand-held instrument for quantitation. At the time this review was written, the test strips and reader were available commercially; however, there was no documentation on performance characteristics of the assay. The assay is purported to detect AFM₁ at the European Union regulatory limit of 0.050 ng/mL in milk and requires 15 min to perform (www.charm.com).

C. Fluorescence Polarization and **Time-Resolved Fluorescence**

Fluorescence polarization (FP) immunoassay has recently been described for a number of mycotoxins, including the aflatoxins, DON, fumonisins, and ZEN (Maragos and Plattner, 2002; Maragos et al., 2001; Nasir and Jolley, 2003). Unlike most of the other sensors described in this review, the FP immunoassays are solution-phase assays: they can be conducted without the attachment of antibody or antigen to a solid surface (Checovich et al., 1995). The principle for FP immunoassay is illustrated in Fig. 3. Fluorescence polarization detectors are indirectly measuring the rate of rotation of a fluorophore in solution. The rate of rotation is directly related to the size of molecules, with larger molecules rotating slower at a given temperature. With FP immunossay a mycotoxin-fluorophore conjugate (tracer) is used. The tracer has a low molecular weight and rotates rapidly in solution. The addition of antitoxin antibody results in the formation of an immune complex of the tracer with the antibody,



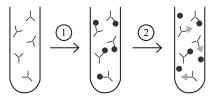


Figure 3. Fluorescence polarization (FP) immunoassay. Assays are performed by placing anti-mycotoxin antibody (Y) into a cuvette followed by: (1) addition of sample extract (toxin: ●) and blanking the instrument, then (2) addition of toxin-fluorophore (tracer: ▲) and incubation, during which the tracer and toxin compete for available antibody. Fluorescence polarization is measured without the need for separating the bound and unbound tracer (see text).

effectively slowing the rate of rotation of the fluorophore and increasing the polarization. The FP immunoassay therefore allows detection of low molecular weight materials in solution without requiring a step to separate the "bound" and "unbound" label, a significant advantage over traditional ELISA techniques.

As with other immunoassays, the selection of appropriate antibody and tracer pairs is essential. While FP immunoassays can be conducted using either the rate of association (kinetic assays) or the endpoint of equilibrated mixture (batch or equilibration assays), in general the latter method may be preferable if untrained personnel will be performing the assays. The time it takes for the antibody/tracer/toxin combination to achieve equilibrium is a critical aspect of FP immunoassays. This equilibration time can vary from 1 min to over 15 min, depending upon the antibody/tracer combination selected. Rapid (30 sec) and sensitive assays for deoxynivalenol were developed using a DON-fluorescein tracer. However, the assay did not come to equilibrium quickly; a potential problem if samples are tested by untrained personnel (Maragos et al., 2002). However, when a different antibody/tracer combination was used, a rapid (1 min) and sensitive assay that quickly came to equilibrium was attained. The latter assay was used to quantitate DON in spiked or naturally contaminated wheat, but not maize, at levels greater than 500 ng/g. Therefore, when appropriate antibody/tracer combinations are used, rapid FP immunoassays can be developed (Maragos and Plattner, 2002). This aspect is important in any competitive immunoassay, but seems to be particularly important with FP immunoassays. The potential speed of FP assays combined with the portability of commercially available devices, suggests this is a promising technology for mycotoxin detection.

Unlike fluorescence polarization immunoassays, time-resolved fluoroimmunoassays (TR-FIA) use the property of fluorescence lifetime. The



rate of decay of fluorescence after a pulse of light at the excitation wavelength differs among different types of fluorophores. The fluorescence lifetime of some fluorophores, such as europium, are much longer than most fluorescent materials present in the matrix as background fluorescence. Therefore, europium can be discriminated from background fluorescence without the need to "blank" the sample. This property is used to advantage in TR-FIAs, which were recently reported for α-zeralanol (zeranol) and α-zearalenol in bovine urine (Cooper et al., 2002). The principle of the assay was essentially that of a competitive ELISA as illustrated in Fig. 1A. An anti-IgG was coated to the microtiter wells, followed by antizearalenol antibody and a zeranol-ovalbumin-europium conjugate. An advantage of the technique was that all the reagents could be adsorbed onto the surface of the wells, eliminating the need for a separate addition/mixing step during the assay. The bovine urine was cleaned up with an immunoaffinity column. The cleaned extract was added to the wells, and plates were shaken and washed (to remove unbound conjugate). An enhancement solution was added, and the europium time-resolved fluorescence was measured. The technique was very sensitive, with limits of detection of 1.3 ng zeranol/mL and 5.6 ng α-zearalenol/mL in bovine urine. A TR-FIA was also developed several years ago for AFB₁ in soya seeds, dried figs, and raisins (Bacigalupo et al., 1994). The limits of detection were 0.5 ng/g, indicating the method was sensitive enough to be used in these commodities.

D. Microbead Assays

Immunoaffinity columns (IAC) have a long history of use in the cleanup of samples for mycotoxin analysis (Scott and Trucksess, 1997). In the most common form, mycotoxin antibodies are coated on the surface of spherical beads and packed in a column. Diluted sample extract is applied to the column. The toxin(s) adhere to the column and most of the potentially interfering material is removed with a wash step. The toxin is then eluted by disrupting the immune-complex, generally by increasing the solvent strength. In many cases the eluted toxin can be derivatized and detected with a portable fluorometer (VICAM LP, Watertown, MA). Alternatively, the eluted toxin can be applied to an instrumental technique for further separation before quantitation. Assays using this concept are available commercially. Microbead assays are miniaturized versions of the IAC assays, often with the cleanup and detection steps performed in a single instrument. Although attaching antibodies to microbeads is the more direct approach, assays can also be conducted with antigen attached to the microbeads in competitive assays similar conceptually to ELISAs (Fig. 4).



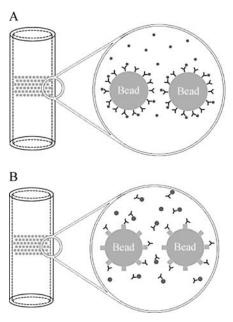


Figure 4. Formats of microbead immunoassays or immunoaffinity columns. (A) Immobilized antibody (Y), to which toxin binds. The beads are then washed and the toxin (●) eluted for detection. (B) Immobilized antigen (■), which competes with the toxin (•) for a limiting amount of antibody (Y) in solution. The capillary is washed and a secondary, labeled, antibody is added (not shown). After a second wash either the labeled antibody is detected directly (in the case of fluorescent labels) or substrate is added to yield a colored product (in the case of enzymatic labels).

Recently, a hand-held microbead sensor was developed for aflatoxins (Carlson et al., 2000). The sensor used a small peristaltic pump to move fluids over a miniaturized affinity column containing aflatoxin antibodies. Samples in buffer solution were allowed to flow over the column, binding the aflatoxin. The column was then washed with buffer and eluted with methanol/water and the aflatoxin measured directly by fluorescence. The fluorescence detector used a xenon arc lamp and a filter to select light at the excitation wavelength of 365 nm. Assays could be conducted within 2 min using a 1-mL sample volume. The fluorescence of aflatoxins is highly sensitive to the presence or absence of the C15-C16 double bond. Aflatoxin B₂ (AFB₂) is more fluorescent than AFB₁. For many chromatographic methods for the aflatoxins, the C15-C16 bond is reacted (e.g., with bromine, iodine, or trifluoroacetic acid) to yield more fluorescent derivatives, increasing the sensitivity of the assay. Commercial affinity



columns also employ a developing (derivatization) solution to enhance fluorescence before detection in portable fluorometers, such as with the Vicam system. The aflatoxin microbead sensor was able to detect 1 mL of a 1.44-ng/mL solution of AFB₂. The range of detection for the device was reported to be 0.1 to 50 ng/mL for aflatoxin in buffer (which aflatoxin was not described). The instrument was capable of holding about 500 mL of reagents, enough for approximately 100 assays before refilling. Given the history of the successful application of commercial IACs, when used in conjuction with a separate fluorometric detection step, the construction of a device combining both steps is a promising development. However, the reuse of antibody-based affinity columns can be problematic. Issues such as fouling of the column, denaturation/renaturation of antibody, and leaching of antibody all impact the degree to which antibody-based affinity columns can be reused.

Affinity columns that have antigen, rather than antibody, bound use a competitive rather than a noncompetitive format. A competitive format has the disadvantage in that the mycotoxin is indirectly detected, but may have an advantage in reusability if the antigen coating is stable. A commercial instrument, KinEXA, available from Sapidyne Instruments (Idaho City, ID), performed many of the functions of an automated IAC assay using microbeads. That instrument was used by Strachan et al. (1997) to develop an application for detection of aflatoxin B₁ in nut puree, peanut butter, and pistachio meal. The assay format is known as sequential injection immunoassay (SIIA), although it has also been called flow injection immunoassay (FIIA). Beads were coated with an AFB₁-BSA conjugate. Assays were conducted by mixing sample with aflatoxin antibody (primary antibody) and pumping this mixture over the beads. A fluorescein-labeled secondary antibody was added and the excess label was washed away. The fluorescence associated with the beads was then determined. Assays were performed in 8 min; however, sample preparation/cleanup required an additional 1 h, which negated some of the potential advantages over ELISA. A similar assay, also using the KinEXA apparatus, was used to detect ZEN standard solutions (Carter et al., 2000). The beads were coated with ZEN-BSA antigen and, as with the aflatoxin assay, a secondary antibody labeled with fluorescein was used. Assay time was described as less than 60 min. The assay was capable of detecting 5 ng ZEN/mL solution; however, because food samples were not examined it is not possible to extrapolate a limit of detection in foods.

The SIIA format has also been applied to AFB₁ using an enzymatic, rather than a fluorescent, label (Garden and Strachan, 2001). The instrumentation was also available commercially, from FIALab Instruments (Bellevue, WA) and uses a small spectrometer, rather than a fluorescence



detector. As with the earlier work the beads were coated with AFB₁-BSA. The secondary antibody was labeled with alkaline phosphatase (rather than fluorescein). A 15-min sample extraction/dilution was combined with a 10-min assay to detect AFB₁ in artificially contaminated pistachios over the range of 4 to 400 ng/g. The minimum concentration of AFB₁ detected was 0.2 ng/mL, equivalent to 4 ng AFB₁/g sample, although matrix interferences were observed.

A variant of the FIIA involves the use of antigen-tagged liposomes instead of microbeads and is known as flow-injection liposome immunoanalysis (FILIA). The format has been applied to analysis of fumonisins in corn (Ho and Durst, 2003). Fumonisin antibodies were coated onto protein A on the capillary walls. Extracts of samples were injected, followed by fumonisin-tagged liposomes. The liposomes were filled with a fluorescent marker (sulforhodamine B). After a wash to remove unbound liposomes, the liposomes were lysed with a detergent and the fluorescence measured. As with an ELISA, the signal from the fluorophore was inversely proportional to the fumonisin concentration. The assays could be performed in 11 min and the limit of detection was 0.1 ng (0.1 mL of a 1 ng FB₁/mL solution) for the FIIA and 2.5 ng for the HPLC-fluorescence method. Recovery of FB₁ from spiked cornmeal ranged from 80% to 92% over the range of 1000 to 4000 ng FB₁/g cornmeal, and the results for spiked samples compared favorably to HPLC. The assay also compared favorably to HPLC for detection of FB1 in commercial corn products and corn-based feeds, suggesting this format has true potential for detecting mycotoxins in foods.

E. Capillary Electrophoretic Immunoassays

While the FIIA and SIIA involve immunoassays conducted in capillaries with microbead or antigen-tagged liposomes, the immunoassays can also be conducted without a supporting phase, e.g., in solution. Capillary electrophoresis (CE) is generally used as a chromatographic technique, where mycotoxins are separated from one another and from matrix components using electrical potential. When used as a chromatographic method, toxins are isolated from food samples using cleanup methods analogous to HPLC, and the cleaned extracts are injected into the capillaries. After separation in an electrical field the analytes are detected, typically using fluorescence or UV absorbance. Capillary electrophoresis as a chromatographic method for mycotoxin analysis has been reviewed (Maragos, 1998).

Recent advances in mycotoxin detection with CE include the use of βcyclodextrins combined with multiphoton excitation for aflatoxin detection (Wei et al., 2000) and the detection of patulin in apple juice by UV (Tsao and



Zhou, 2000). The former is a very sensitive method, having concentration detection limits for aflatoxins in the range of 0.2 to 0.4 nM (0.06 to 0.13 ng/ mL). A method combining the detection of ochratoxins A and B with that of four aflatoxins has also been described (Peña et al., 2002). Micellar electrokinetic capillary chromatography (MECC) is a variant of capillary zone electrophoresis that is particularly useful for detecting neutral compounds, such as the aflatoxins. An MECC-based method using an electronically controlled injection process (vs. the more generally used pressure or electrokinetic injection) for measurement of aflatoxin standard solutions was described (Dickens and Sepaniak, 2000). The injection platform is interesting because it resembles those used in microchip formats. The reported limits of detection (S/N of 2) of aflatoxins in buffer ranged from 7.2 nM (2.3 ng/mL) for AFB₂ to 31 nM (10 ng/mL) for AFG₁.

The combination of CE with immunoassay (Fig. 5) has also been used for analysis of fumonisins in maize (Maragos, 1997). After extraction of corn with water, a portion of the extract was mixed with antibody and a fluorescein-tagged fumonisin (tracer). Fumonisin in the sample competed with the tracer for binding to the antibody. Application of voltage resulted in a separation of the bound and unbound tracer. With increasing fumonisin in the sample the level of bound tracer decreased and the level of unbound tracer increased, signaling the presence of the toxin. The assays were relatively rapid, using a 4-min electrophoretic step and a 2-min wash of the capillary between samples. The sensitivity of the technique was highly

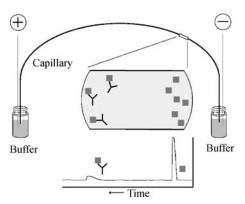


Figure 5. Schematic of one form of capillary electrophoretic immunoassay. In this format antibody (Y) is combined with sample and a fluorescently labeled toxin (tracer: **1**). Bound and unbound tracer are separated in an electric field. In the presence of free toxin the amount of unbound tracer increases and the amount of bound tracer decreases, changing the relative sizes of the two peaks.



dependent upon the concentration of antibody used. At the optimum antibody concentration, the midpoint for the calibration curves ranged from 500 to 1700 ng FB₁/mL. Unfortunately this level of sensitivity was only sufficient for analyzing maize samples containing substantial concentrations of FB₁ (greater than 10,000 ng/g). Sensitivity in this format is likely highly dependent upon the relative affinity of the antibody for the fumonisin and the tracer under conditions of high electric field strength, which might be improved with a different fumonisin antibody.

Other, less well developed, techniques also use capillaries to deliver reagents to multichannel devices, with the goal of multianalyte detection (Narang et al., 1998). It is likely the improvements in microfluidics and miniaturization of sensor components will lead to the development of multichannel mycotoxin sensors, perhaps using "sensor on-a-chip" technologies, or array biosensors for mycotoxin detection (Rowe-Taitt et al., 2000).

III. FUTURE CHALLENGES

Sensitive and reproducible quantitation of analytes present at low levels in complex matrices begets certain challenges in the development of assays. Principally, these include ensuring that the sample being tested is representative of the larger lot, and that the assays are sensitive enough and accurate enough for their intended use. The state of mycotoxin analysis is that, for most of the known mycotoxins current analytical techniques have overcome these obstacles. For the lesser known mycotoxins and for those mycotoxins that remain to be discovered, the challenges are to develop methods that can be used for routine analysis in laboratories. For most of the known mycotoxins the remaining challenges are to render the analytical process more efficient through the application of new technologies that may be less labor intensive and therefore less expensive.

For laboratory-based assays an on-going challenge has been the simultaneous detection of multiple toxins. Despite the efforts of researchers throughout the years to develop multiple mycotoxin assays, the need still exists for the detection of multiple families of mycotoxins from the same sample. For example, the potential co-occurrence of aflatoxins and fumonisins in corn suggests screening for both groups of mycotoxins is warranted. Yet, the differences in the polarity of these toxins, the differences in their physical properties (fluorescence, UV absorbance, or lack thereof), and the concentration ranges of interest (ng/g vs. µg/g) have made simultaneous detection difficult. In this regard, the mass spectrometric methods hold considerable promise, as do those that use chromatography combined with multiple detectors.



Rapid detection of multiple toxins is also an area where emerging technologies for mycotoxin detection may find use. It is not difficult to envision antibody-based screening assays for multiple mycotoxins using array formats similar to those described for higher molecular weight toxins (Rowe-Taitt et al., 2000). The continued miniaturization of analytical instrumentation also opens possibilities for hand-held devices that perform the same duties as today's benchtop devices. This is particularly true for instruments measuring absorbance or fluorescence. The ongoing development of microfluidics and capillary-based assays (capillary electrophoresis, FIIA, SIIA, etc.) suggests that the development of assay-on-a-chip technology is in the near future.

Biosensors can also be constructed using binding events other than those of antibody/antigen. An example are the flow injection assays developed for aflatoxin M₁ in milk based upon lipid bilayer membranes (Andreou and Nikolelis, 1998; Siontorou et al., 1998). In two formats the AFM₁ was shown to interact directly with the bilayers and to inhibit the hybridization of single-stranded DNA oligomers. The perturbations in the bilayer were measured electrochemically. In the first format the working range of AFM₁ standards was 1.9 to 20.9 nM (0.62 to 6.9 ng/mL), while in the second the limit of detection was 0.5 nM (0.16 ng/mL) and the assays were very rapid: less than 1 min (Siontorou et al., 1998). In the non-DNA hybridization format, and using filter-supported bilayer membranes, AFM₁ was shown to alter the phase transition temperature of the membrane (Andreou and Nikolelis, 1998). Detection using the electrochemical response was very rapid (10 sec), and the assays had subnanomolar limits of detection. By adjusting the flow rate through the membranes, effects due to potential matrix interferences such as casein were minimized (Andreou and Nikolelis, 1998). Milk and milk products spiked with AFM₁ showed a good agreement between the spiking level and the concentration detected in the range of 3.8 to 14.6 nM AFM₁ and at a rate of four samples per min.

While the focus of this review has been on antibody-based technologies, the use of biologically derived binding elements is not inherently essential to the development of functional sensors (although, by definition it is required for a biosensor). The improvement of antibodies and antibody fragments through recombinant techniques is one approach to improving immunoassay (Yuan et al., 2000; Zhou et al., 1996). An alternative is the development of nonbiologically based binding and transduction elements. An example of the latter are the molecularly imprinted polymers (MIPS). Usually MIPS are synthesized using a template molecule similar in structure to the analyte. Functional monomers that interact with the template noncovalently are polymerized together using cross-linking monomers. The entrapped template is then removed and the polymer used to bind



the analyte. Functional monomers that interact covalently with the template or analyte can also be used.

High affinity MIPS could essentially perform the same functions as antibodies in immunoassays and might benefit from greater solvent tolerance or tolerance to extremes of pH or ionic strength. Recently, MIPS have been reported for the ochratoxins (Jodlbauer et al., 2002), DON, and ZEN (Weiss et al., 2003). While the affinities are not yet competitive with those of antibodies, there is excellent potential for further development.

As discussed at the beginning of this review, further progress can be expected on technologies that detect mycotoxigenic fungi as well. Detection of the fungi may involve detection of physical changes to the commodity (as with the previously mentioned color, fluorescence, and IR methods, and machine vision systems). The growth of fungi also causes chemical changes, some of which may be detectable by optical methods, or by alternative methods measuring changing volatile composition such as GC-MS or electronic noses (Keshri and Magan, 2000; Olsson et al., 2000, 2002). Furthermore, the detection of DNA of mycotoxigenic fungi is possible, using a variety of polymerase chain reaction (PCR)-based methods such as random amplification of polymorphic DNA (RAPD), reverse transcription (RT)-PCR, competitive PCR, and real-time quantitative PCR. The latter techniques, with regard to detection of mycotoxigenic fungi, were recently reviewed (Edwards et al., 2002).

The aforementioned emerging technologies for mycotoxin detection are at various stages in the progression to useful analytical tools. Some have been tested and found, at least currently, to be poor candidates for further development. Some are advanced enough for field use. Many still face the challenge of making the transition from proof-of-concept assays using toxins in buffer solutions to analysis of food samples. Others have been demonstrated to work with food samples, but still face the challenges of ease of use and validation by multiple laboratories. Despite these obstacles, detection technologies continue to advance, and the prospects for further improvements in mycotoxin detection methodology are excellent.

REFERENCES

Andreou, V., Nikolelis, D. (1998). Flow injection monitoring of aflatoxin M₁ in milk and milk preparations using filter-supported bilayer lipid membranes. Anal. Chem. 70:2366-2371.

Bacigalupo, M. A., Ius, A., Meroni, G., Dovis, M., Petruzzelli, E. (1994). Determination of aflatoxin B₁ in agricultural commodities by timeresolved fluoroimmunoassay and immunoenzymometric assay. Analyst 119:2813-2815.



- Bhattacharya, D., Bhattacharya, R., Dhar, T. K. (1999). A novel signal amplification technology for ELISA based on catalyzed reporter deposition. Demonstration of its applicability for measuring aflatoxin B₁. J. Immunol. Meth. 230:71–86.
- Cappiello, A., Famiglini, G., Tirillini, B. (1995). Determination of aflatoxins in peanut meal by 1 c/ms with a particle beam interface. Chromatographia 40:411-416.
- Carlson, M. A., Bargeron, C. B., Benson, R. C., Fraser, A. B., Phillips, T. E., Velky, J. T., Groopman, J. D., Strickland, P. T., Ko, H. W. (2000). An automated, handheld biosensor for aflatoxin. Biosen. Bioelect. 14:841-848.
- Carter, R. M., Jacobs, M. B., Lubrano, G. J., Guilbault, G. G. (1997). Rapid detection of aflatoxin B₁ with immunochemical optrodes. Anal. Lett. 30:1465-1482.
- Carter, R. M., Blake, R. C. II, Mayer, H. P., Echevarria, A. A., Nguyen, T. D., Bostanian, L. A. (2000). A fluorescent biosensor for detection of zearalenone. Anal. Lett. 33:405-412.
- Checovich, W. J., Bolger, R. E., Burke, T. (1995). Fluorescence polarization—a new tool for cell and molecular biology. *Nature* 375:254–256.
- Cooper, K. M., Tuomola, M., Lahdenperä, S., Lövgren, T., Elliott, C. T., Kennedy, D. G. (2002). Development and validation of dry reagent time-resolved fluoroimmunoassays for zeranol and α-zearalenol to assist in distinguishing zeranol abuse from Fusarium spp. toxin contamination in bovine urine. Food Addit. Contam. 19:1130-1137.
- Daly, S. J., Keating, G. J., Dillon, P. P., Manning, B. M., O'Kennedy, R. O., Lee, H. A., Morgan, M. R. A. (2000). Development of surface plasmon resonance-based immunoassay for aflatoxin B₁. J. Agric. Food Chem. 48:5097-5104.
- Daly, S. J., Dillon, P. P., Manning, B. M., Dunne, L., Killard, A., O'Kennedy, R. O. (2002). Production and characterization of murine single chain Fv antibodies to aflatoxin B₁ derived from a pre-immunized antibody phage display library system. Food Agric. Immunol. 14:255-274.
- De Saeger, S., Van Peteghem, C. (1996). Dipstick enzyme immunoassay to detect Fusarium T-2 toxin in wheat. Appl. Environ. Microbiol. 62:1880-1884.
- De Saeger, S., Van Peteghem, C. (1999). Flow-through membrane-based enzyme immunoassay for rapid detection of ochratoxin A in wheat. J. Food Prot. 62:65-69.
- De Saeger, S., Sibanda, L., Desmet, A., Van Peteghem, C. (2002). A collaborative study to validate novel field immunoassay kits for rapid mycotoxin detection. Int. J. Food Microbiol. 75:135-142.



Dickens, J., Sepaniak, M. (2000). Modular separation-based fiber-optic sensors for remote in situ monitoring. J. Environ. Monit. 2:11–16.

- Dowell, F. E., Ram, M. S., Seitz, L. M. (1999). Predicting scab, vomitoxin, and ergosterol in single wheat kernels using near-infrared spectroscopy. Cereal Chem. 76:573-576.
- Dowell, F. E., Pearson, T. C., Maghirang, E. B., Xie, F., Wicklow, D. T. (2002). Reflectance and transmittance spectroscopy applied to detecting fumonisin in single corn kernels infected with Fusarium verticillioides. Cereal Chem. 79:222-226.
- Edwards, S. G., O'Callaghan, J., Dobson, A. D. W. (2002). PCR-based detection and quantification of mycotoxigenic fungi. Mycol. Res. 106:1005-1025.
- Garden, S. R., Strachan, N. J. C. (2001). Novel colorimetric immunoassay for the detection of aflatoxin B₁. Anal. Chim. Acta 444:187–191.
- Hartl, M., Humpf, H.-U. (1999). Simultaneous determination of fumonisin B₁ and hydrolyzed fumonisin B₁ in corn products by liquid chromatography/electrospray ionization mass spectrometry. J. Agric. Food Chem. 47:5078-5083.
- Hirano, S., Okawara, N., Narazaki, S. (1998). Near infra red detection of internally moldy nuts. *Biosci. Biotechnol. Biochem.* 62:102–107.
- Ho, J. A. A., Wauchope, R. D. (2002). A strip liposome immunoassay for aflatoxin B₁. Anal. Chem. 74:1493-1496.
- Ho, J. A., Durst, R. A. (2003). Detection of fumonisin B₁: comparison of flow-injection liposome immunoanalysis with high-performance liquid chromatography. Anal. Biochem. 312:7-13.
- Jodlbauer, J., Maier, N. M., Lindner, W. (2002). Towards ochratoxin A selective molecularly imprinted polymers for solid-phase extraction. J. Chromatogr., A 945:45-63.
- Karuna, R., Sashidhar, R. B. (1999). Use of ion-exchange chromatography coupled with TLC-laser scanning densitometry for the quantitation of fumonisin B₁. Talanta 50:381-389.
- Keshri, G., Magan, N. (2000). Detection and differentiation between mycotoxigenic and non-mycotoxigenic strains of two Fusarium spp. using volatile production profiles and hydrolytic enzymes. J. Appl. Microbiol. 89:825-833
- Kos, G., Lohninger, H., Krska, R. (2002). Fourier transform mid-infrared spectroscopy with attenuated total reflection (FT-IR/ATR) as a tool for the detection of Fusarium fungi on maize. Vib. Spec. 29:115-119.
- Liedberg, B., Nylander, C., Lundström, I. (1995). Biosensing with surface plasmon resonance-how it all started. Biosen. Bioelect. 10:i-ix.
- Maragos, C. M. (1997). Detection of the mycotoxin fumonisin B₁ by a



- combination of immunofluorescence and capillary electrophoresis. Food Agric. Immunol. 9:147-157.
- Maragos, C. M. (1998). Analysis of mycotoxins with capillary electrophoresis. Sem. Food Anal. 3:353-373.
- Maragos, C. M., Thompson, V. S. (1999). Fiber-optic immunosensor for mycotoxins. Nat. Toxins 7:371-376.
- Maragos, C. M., Plattner, R. D. (2002). Rapid fluorescence polarization immunoassay for the mycotoxin deoxynivalenol in wheat. J. Agric. Food Chem. 50:1827-1832.
- Maragos, C. M., Jolley, M. E., Plattner, R. D., Nasir, M. S. (2001). Fluorescence polarization as a means for determination of fumonisins in maize. J. Agric. Food Chem. 49:596-602.
- Maragos, C. M., Jolley, M. E., Nasir, M. S. (2002). Fluorescence polarization as a tool for the determination of deoxynivalenol in wheat. Food Additives and Contaminants 19:400–407.
- McClure, W. F., Farsaie, A. (1980). Dual-wavelength fiber optic photometer measures fluorescence of aflatoxin contaminated pistachio nuts. Trans. ASAE, 204-207.
- Moghaddam, A., Løbersli, I., Gebhardt, K., Braunagel, M., Marvik, O. J. (2001). Selection and characterization of recombinant single-chain antibodies to the hapten aflatoxin-B₁ from naive recombinant antibody libraries. J. Immunol. Meth. 254:169-181.
- Mullett, W., Lai, E. P. C., Yeung, J. M. (1998). Immunoassay of fumonisins by a surface plasmon resonance biosensor. *Anal. Biochem.* 258:161–167.
- Musser, S. M., Eppley, R. M., Trucksess, M. W. (2002). Electrospray mass spectrometry for fumonisin detection and method validation. In: DeVries, J. W., Trucksess, M. W., Jackson, L. S., eds. Mycotoxins and Food Safety. New York: Kluwer Academic, pp. 95-105.
- Narang, U., Gauger, P. R., Kusterbeck, A. W., Ligler, F. S. (1998). Multianalyte detection using a capillary-based flow immunosensor. Anal. Biochem. 255:13-19.
- Nasir, M. S., Jolley, M. E. (2003). Fluorescence polarization (FP) assays for the determination of grain mycotoxins (fumonisins, DON vomitoxin and aflatoxins). Comb. Chem. High Throughput Screen. 6:267-273.
- Olsson, J., Börjesson, T., Lundstedt, T., Schnürer, J. (2000). Volatiles for mycological quality grading of barley grains: determinations using gas chromatography-mass spectrometry and electronic nose. *Intl. J. Food* Microbiol. 59:167-178.
- Olsson, J., Börjesson, T., Lundstedt, T., Schnürer, J. (2002). Detection and quantification of ochratoxin A and deoxynivalenol in barley grains by GC-MS and electronic nose. Int. J. Food Microbiol. 72:203-214.



Pearson, T. (1996). Machine vision system for automated detection of stained pistachio nuts. Lebensmitelw. U. Technol. 29:203-209.

- Pearson, T., Wicklow, D. T., Maghirang, E. B., Xie, F., Dowell, F. E. (2001). Detecting aflatoxin in single corn kernels by transmittance and reflectance spectroscopy. Trans. ASAE 44:1247-1254.
- Peña, R., Alcaraz, M. C., Arce, L., Ríos, A., Valcárcel, M. (2002). Screening of aflatoxins in feed samples using a flow system coupled to capillary electrophoresis. J. Chromatogr. A 967:303–314.
- Pharmacia Biosensor. (1995). BIAtechnology Note 101 Surface Plasmon Resonance, A Description of the Detecting Principle. S-751 82 Uppsala, Sweden: Pharmacia Biosensor AB.
- Plattner, R. D., Maragos, C. M. (2003). Determination of deoxynivalenol and nivalenol in corn and wheat by liquid chromatography with electrospray mass spectrometry. J. Assoc. Off. Anal. Chem. Internat. 86:61-
- Rowe-Taitt, C. A., Golden, J. P., Feldstein, M. J., Cras, J. J., Hoffman, K. E., Ligler, F. S. (2000). Array biosensor for detection of biohazards. Biosen. Bioelectron. 14:785-794.
- Ruan, R., Ning, S., Song, A., Ning, A., Jones, R., Chen, P. (1998). Estimation of *Fusarium* scab in wheat using machine vision and a neural network. Cereal Chem. 75:455-459.
- Schneider, E., Usleber, E., Märtbauer, E. (1995a). Rapid detection of fumonisin B₁ in corn-based food by competitive direct dipstick enzyme immunoassay/enzyme-linked immunofiltration assay with integrated negative control reaction. J. Agric. Food Chem. 43: 2548-2552.
- Schneider, E., Usleber, E., Märtlbauer, E., Dietrich, R., Terplan, G. (1995b). Multimycotoxin dipstick enzyme immunoassay applied to wheat. Food Addit. Contam. 12:387-393.
- Schnerr, H., Vogel, R. F., Niessen, L. (2002). A biosensor-based immunoassay for rapid screening of deoxynivalenol contamination in wheat. Food Agric. Immunol. 14:313-321.
- Scott, P. M., Trucksess, M. W. (1997). Application of immunoaffinity columns to mycotoxin analysis. J. Assoc. Off. Anal. Chem. Internat. 80:941-949.
- Shephard, G. S. (2001). Analytical methodology for mycotoxins: recent advances and future challenges. In: de Koe, W. J., Samson, R. A., van Egmond, H. P., Gilbert, J., Sabino, M., eds. Mycotoxins and Phycotoxins in Perspective at the Turn of the Millennium. 1st ed. Wageningen: Ponsen & Looyen, pp. 19–28.
- Siontorou, C. G., Nikolelis, D. P., Miernik, A., Krull, U. J. (1998). Rapid methods for detection of aflatoxin M₁ based on electrochemical



- transduction by self-assembled metal-supported bilayer lipid membranes (s-BLMs) and on interferences with transduction of DNA hybridization. *Electrochim. Acta* 43:3611–3617.
- Strachan, N. J. C., John, P. G., Miller, I. G. (1997). Application of an automated particle-based immunosensor for the detection of aflatoxin B₁ in foods. Food Agric. Immunol. 9:177–184.
- Stroka, J., Anklam, E. (2000). Development of a simplified densitometer for the determination of aflatoxins by thin-layer chromatography. J. Chromatogr., A 904:263-268.
- Sydenham, E. W., Shephard, G. S. (1997). Chromatographic and allied methods of analysis for selected mycotoxins. In: Gilbert, J., ed. Progress in Food Contaminant Analysis. New York: Chapman and Hall, pp. 65–146.
- Tanaka, T., Yoneda, A., Sugiura, Y., Inoue, S., Takino, M., Tanaka, A., Shinoda, A., Suzuki, H., Akiyama, H., Toyoda, M. (2002). An application of liquid chromatography and mass spectrometry for determination of aflatoxins. Mycotoxins 52:107-113.
- Thompson, R. B., Ligler, F. S. (1991). Chemistry and technology of evanescent wave biosensors. In: Wise, D. L., Wingard, L. B., eds. Biosensors with Fiberoptics. Clifton, NJ: Humana Press, Inc., pp. 111–138.
- Thompson, V. S., Maragos, C. M. (1996). Fiber-optic immunosensor for the detection of fumonisin B₁. J. Agric. Food Chem. 44:1041–1046.
- Trucksess, M. W. (2001). Rapid analysis (thin layer chromatographic and immunochemical methods) for mycotoxins in foods and feeds. In: de Koe, W. J., Samson, R. A., van Egmond, H. P., Gilbert, J., Sabino, M., eds. Mycotoxins and Phycotoxins in Perspective at the Turn of the Millennium. 1st ed. Wageningen: Ponsen & Looyen, pp. 29-40.
- Tsao, R., Zhou, T. (2000). Micellar electrokinetic capillary electrophoresis for rapid analysis of patulin in apple cider. J. Agric. Food Chem. 48:5231-5235.
- Tüdos, A. J., Lucas-van den Bos, H. R., Stigter, E. C. A. (2003). Rapid surface plasmon resonance-based assay of deoxynivalenol (DON). J. Agric. Food Chem. 51:5843-5848.
- van der Gaag, B., Burggaaf, R. A., Wahlström, L. (1996). Application and development of a BIAcore® for the detection of mycotoxins in food and feed. In: Poster Presentation at the 110th AOAC International Annual Meeting and Exposition. Orlando, FL, Sept 8-12.
- van der Gaag, B., Spath, S., Dietrich, H., Stigter, E., Boonzaaijer, G., van Osenbruggen, T., Koopal, K. (2003). Biosensors and multiple mycotoxin analysis. Food Control 14:251-254.
- Wei, J., Okerberg, E., Dunlap, J., Ly, C., Shear, J. B. (2000). Determination



of biological toxins using capillary electrophoretic chromatography with multiphoton-excited fluorescence. Anal. Chem. 72:1360-1363.

- Weiss, R., Freudenschuss, M., Krska, R., Mizaikoff, B. (2003). Improving methods of analysis for mycotoxins: molecularly imprinted polymers for deoxynivalenol and zearalenone. Food Addit. Contam. 20:386-395.
- Yuan, Q., Hu, W., Pestka, J. J., He, S. Y., Hart, L. P. (2000). Expression of a functional antizearalenone single-chain fv antibody in transgenic Arabidopsis plants. Appl. Environ. Microbiol. 66:3499-3505.
- Zhou, H.-R., Pestka, J. J., Hart, L. P. (1996). Molecular cloning and expression of recombinant phage antibody against fumonisin B₁. J. Food Prot. 59:1208-1212.
- Zöllner, P., Jodlbauer, J., Lindner, W. (1999). Determination of zearalenone in grains by high-performance liquid chromatography-tandem mass spectrometry after solid-phase extraction with RP-18 columns or immunoaffinity columns. J. Chromatogr. A 858:167-174.

